Short Communication

Cation Effects on the Fluorescence of Isolated Chloroplasts

Peter H. Homann

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

At room temperature, the fluorescence of chloroplasts *in vivo* and *in vitro* originates nearly exclusively from the chlorophyll of the oxygen evolving system II of the photosynthetic apparatus. The yield and the kinetics of this light emission are closely coupled to the primary photochemical events of photosystem II. Fluorescence studies, therefore, have contributed much to our knowledge about the water oxidizing photoact. According to Duysens and Sweers (3) the fluorescence yield is determined by the oxidation state of the primary electron acceptor Q: when Q is oxidized, the fluorescence is quenched and, consequently, a low yield is observed; since reduced $Q(Q^-)$ does not quench, a maximal fluorescence yield indicates a complete reduction of the electron acceptor pools containing Q. Several anomalies in the fluorescence kinetics of intact algae have led to the assumption of an non-homogeneous pool Q containing, even after the primary activation step (8), active and inactive oxidants Q_a and Q_i' (1, 2, 4, 12). Both forms of Q are thought to quench the fluorescence in their oxidized states, albeit with slightly different effectiveness. A photoreduction of the total pool Q requires an activation of any Q_i' to Q_a, which according to some authors (1, 4, 12) is sensitized by photosystem I.

In this communication, we wish to report on an overriding influence of the ionic environment on the fluorescence yield of isolated chloroplasts. It will be shown that the fluorescence yield can be changed at will simply by varying the composition of the suspension medium. Accordingly, the fluorescence yield may possibly be governed not only by the oxidation state of the Q-pool, but also by ion induced structural changes.

Results and Discussion

The preparation of the chloroplasts from plants grown in an un-airconditioned greenhouse, and the methods used for the measurement of the fluorescence, have been described earlier (5). The effect of salt additions on the fluorescence yield was discovered when the light emission was always found to be higher after uncoupling the electron transport from photophosphorylation by the addition of methylamine HCl (pH 7.3). It was then observed that much larger increases of the fluorescence yield could be obtained after addition of Na-salts or, in particular, Mg-salts (Figs. 1, 2, 3).

For an evaluation of this "salt effect", it is necessary to discuss briefly the normal characteristics of the chloroplast fluorescence. When the light is turned on, one observes a fast rise of the fluorescence emission to a low level F_0. Subsequently, the fluorescence increases in a typical biphasic manner (3, 10) to a steady state level $F\infty$. This rise lasts for a few sec, and reflects the reduction of the pool of primary electron acceptors (3). The "basic fluorescence" (F_0) on the other hand, does not depend on the oxidation state of this pool. However, it ought to be quite high when the "quencher" Q is disengaged from the pigment system II, for example as a consequence of a structural disorganization of the membrane system in the chloroplast. This has actually been observed (5). The steady state level $F\infty$ marks an equilibrium of the rate of the system II mediated photoreduction of the Q-pool, and the rate of its reoxidation by oxygen or photosystem I. In the presence of DCMU, this reoxidation is inhibited, and complete photoreduction is accomplished. Depending on the electron donating capacity of photosystem II, the electron abstracting ability of system I, and the light intensity, the level $F\infty$ is equal to, or lower than, the level F_{DCMU}. In most chloroplast preparations there exists a portion of Q which appears to be associated with inactive system II units, and, consequently, cannot be photoreduced. Following a chemical reduction with dithionite, the fluorescence level is increased over the level F_{DCMU} to a maximal yield F_{red}, probably in proportion to this additional amount of Q (5, 6).

Fig. 1 and 2 unequivocally show that the overall increase of the fluorescence emission after addition of Mg-ions is nearly exclusively due to a change in the yield of that portion of the fluorescence which is controlled by the oxidation state of Q. In other words, the levels $F\infty$, F_{DCMU} and F_{red} are increased,
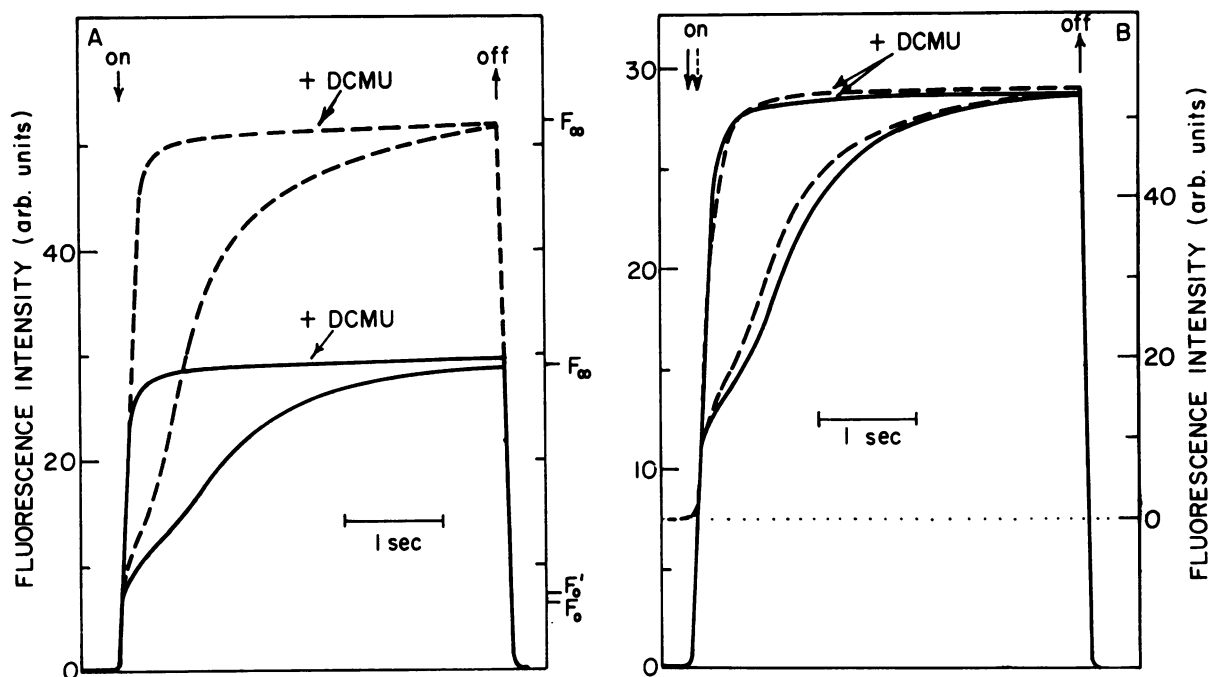
Fig. 1. Effect of 3.3 mM $MgCl_2$ on the fluorescence of whole chloroplasts isolated from *Phytolacca americana*. 5.5 μM chl in 0.4 M sucrose containing 0.05 M tris HCl pH 7.3 and 0.01 M NaCl. T $=$ 17°; exciting light for the fluorescence at 684 nm: 7.5 n einst·sec^{-1} cm^{-2}, λ_{max} 570 nm. A new chloroplast sample was used for each recording. Restoration was achieved by a 1 min illumination with 1.4 n einst·sec^{-1} cm^{-2} far-red light, λ_{max} 717 nm (ref. 5). After addition of $MgCl_2$, 3 min incubation in the dark were necessary for a complete development of the "salt effect". 10 μM DCMU were added as indicated to "restored" chloroplast 1 min prior to the recording. No Mg^{2+} added:———; Mg^{2+} present: - - -. Rate of ferricyanide reduction in the actinic beam (μmoles ferricyanide reduced per mg chlorophyll per hr): No $MgCl_2$: 60; $+$ $MgCl_2$: 72. A) fluorescence curves recorded with constant sensitivity setting; B) fluorescence curves recorded with different sensitivity settings to obtain equal deflection for the variable fluorescence in the absence (left ordinate) and the presence of $MgCl_2$ (right ordinate).

whereas the level F_o is not, or only very little affected.

From Fig. 3 it can be seen that our new "salt effect" depends on the concentration of the type of cation added, rather than on the concentration of the anion, and that Mg-ions are much more effective than Na-ions. The influence of Li$^+$ and K$^+$ was similar to that shown for Na$^+$, but the response of the fluorescence to the addition of Ca^{2+} appeared to be quite complex: low concentrations increased the fluorescence yield, but when more Ca-ions were added, a decrease was observed. Cu-ions, on the other hand, quenched the variable fluorescence F∞-F$_o$ markedly. This finding did not come as a surprise, since Cu^{2+} has long been known to inhibit photosynthetic electron transport (11). It should be remembered that the influence of added Mn-ions is of a completely different kind. We have reported earlier that Mn^{2+} restores the fluorescence rise in inactivated, manganese-depleted chloroplasts, presumably by donating electrons to the reaction center of system II. The levels F_{DCMU} and F_{red}, however, are not affected (6).

The magnitude of the cation induced increase of the fluorescence yield was different with each chloroplast preparation and, during the winter months, was

found to be very low with chloroplasts from green tobacco. When in our usually employed suspension medium containing 0.4 M sucrose, 10 mM NaCl and 50 mM tris-HCl (pH 7.3) the tris buffer was replaced by tricine-NaOH buffer, the effect was always present (Fig. 2). In general, the "salt effect" could be expected to be large, when the ratio F_{DCMU}/F_o was low in the absence of an added salt. With tricine-NaOH as the buffer, this was always the case (table I).

The question could be asked whether Mg-ions had any influence on the Hill activity. When the ferricyanide photoreduction was measured under the conditions used for the fluorescence recordings, the rate always turned out to be slightly higher in the presence of Mg^{2+}. However, many experiments, a selection of which is shown in table II, failed to give a clear correlation between the increase of the Hill rate and the increase of the variable fluorescence F∞-F$_o$. Preliminary experiments also revealed that the relative increase of F_{DCMU} was the same at 7.5 and 0.75 n einst sec^{-1} cm^{-2} incident light intensity, and that it did not vary significantly, if at all, with the wavelength of the exciting light between 405 and 631 nm.

Table I. *Mg²⁺ Induced Changes of the Variable Fluorescence Yield From "Low-salt" and "High-salt" Chloroplasts*

Suspension medium of chloroplasts (5 5 μM chlorophyll) contained 0.4 M sucrose, 10 mM NaCl and 50 mM buffer pH 7.3 as indicated. All other experimental conditions see legend of Fig. 1. The determinations were made during December 1968 (see text).

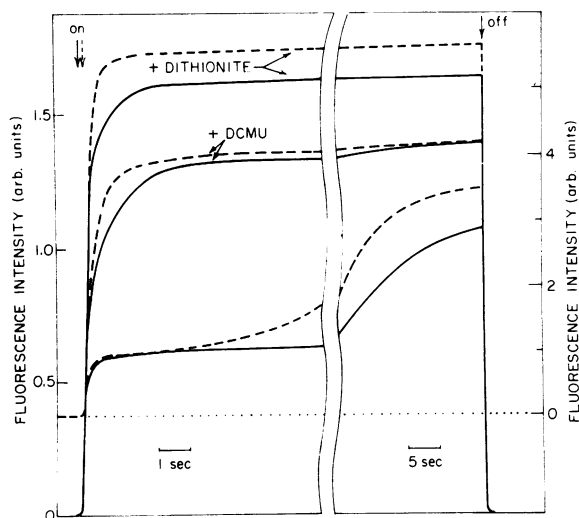| Plant material	Buffer	F_{DCMU}/F_0	
		—MgSO₄	+12 mM MgSO₄
Nicotiana tabacum NC95	tris-HCl	3.1	3.1
	tricine-NaOH	2.4	3.6
Nicotiana tabacum NC95	tris-HCl	4.1	4.1
	tricine-NaOH	2.6	4.8
Spinacca oleracca	tris-HCl	3.2	3.2
	tricine-NaOH	2 3	4.0
Nicotiana tabacum NC95 (sonicated chloroplasts)	tricine-NaOH	2.8	2.9

FIG. 2. Effect of 16 mM MgSO₄ on the fluorescence of chloroplasts from *Nicotiana tabacum*, var. NC 95, at a low light intensity (0.75 n einst·sec⁻¹ cm⁻², λ_max 570 nm). Chloroplasts were suspended in 0.4 M sucrose, 0.05 M tricine NaOH, and 0.01 NaCl. The sensitivity of the apparatus was adjusted to give an equal deflection F_{DCMU}-F_0 for the fluorescence in the absence (——, left ordinate) and presence of MgSO₄ (- - -, right ordinate). T = 20°. Other experimental conditions, see legend of Fig. 1. DCMU was added to "restored" chloroplasts, dithionite was added after the DCMU addition without a far-red preillumination.

At present it is difficult to give a satisfactory explanation of our data. It is possible that Mg-ions facilitate an activation of Q_i' to Q_a, thus allowing a more complete photoreduction of the primary oxidants. Since the salt addition changes F_{red} (Fig. 2), such a hypothesis would require the additional assumption that either Q_i cannot be reduced by dithionite, or that Q_i' quenches the fluorescence like Q_i'. Moreover, when the amount of primary oxidants was estimated by the ferricyanide-titration technique of Malkin and Kok (10), no change of the pool size was observed (Fig. 4). It can be argued that this procedure gives an estimate for the size of

the total pool of electron acceptors, which consists of rather small amounts of Q, and comparatively much larger pools of the secondary oxidant "A" (8,9). Any change of Q only would probably not be detected by the titration technique. For this reason we also compared the fluorescence curves in the presence of DCMU. The areas over these curves before and after addition of Mg²⁺ should indicate more precisely any difference in the size of the small pools Q (9). Fig. 1B and 2 allow such a comparison under 2 different experimental conditions. In these experiments the fluorescence traces had been normalized in respect to the levels F_0 and F_{DCMU} by varying the sensitivity setting of our fluorescence detector. These experiments were inconclusive with our systems, mainly because Mg²⁺ not only increased the variable fluorescence yield, but also speeded up the fluorescence rise. This was seen best at low light intensities (Fig. 2).

Whatever relation exists between the increased fluorescence yield and the amount of photoreduced Q, we feel at this point that the main reason for the change in the light emission is a cation induced
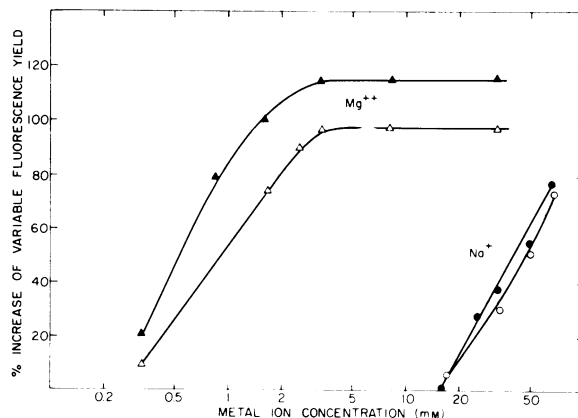

FIG. 3. Dependence of the cation induced increase of the variable fluorescence on the concentration of the added ion. Experimental conditions, see legend of Fig. 1. Chloroplasts from green tobacco NC 95. ●,▲: salts added as chlorides; ○,△: salts added as sulfates.

Table II. *Effect of Mg^{2+} on the Fluorescence Intensity and on the Hill Reaction*

Suspension medium of chloroplasts (5.5 μM chlorophyll for fluorescence, 11 μM for Hill reaction) contained 0.4 M sucrose, 10 mM NaCl, 18 mM methylamine HCl pH 7.3, and 50 mM buffer (pH 7.3) as indicated. Mg^{2+} was added as 3.3 mM $MgCl_2$, or 16 mM $MgSO_4$ in the experiments with tobacco NC95. The temperature was 20°; all other experimental conditions see legend of Fig. 1. For determination of Hill activity (R = rate in μmoles ferricyanide reduced per mg chlorophyll per hr) 0.33 mM ferricyanide was added, and the sample illuminated for 4 min in the fluorescence apparatus. F_o', $F\infty'$ and R′ represent the values in the presence of Mg^{2+}.

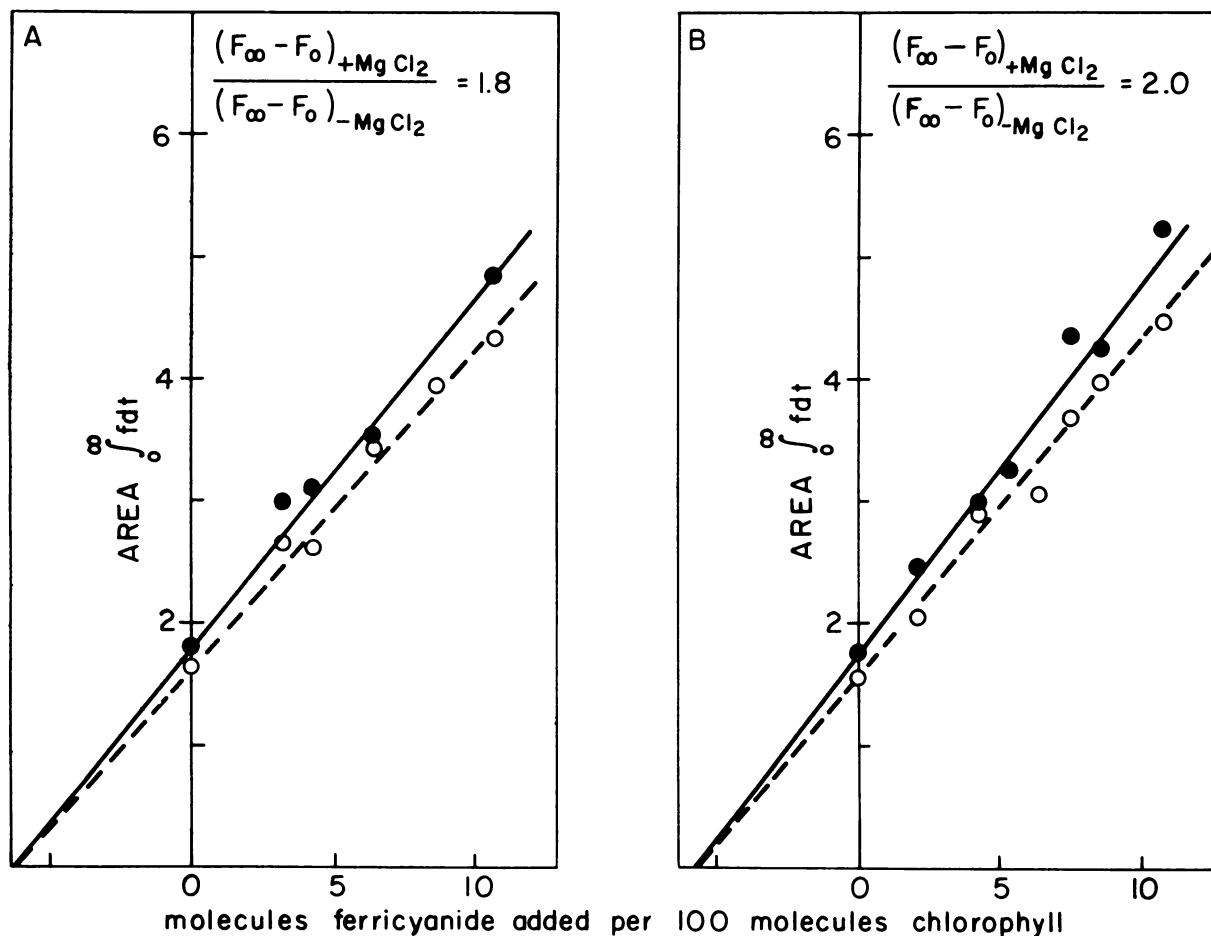| Source of chloroplasts	Buffer	Fluorescence		Hill activity R′/R
		F_o'/F_o	$(F\infty'-F_o')/(F\infty-F_o)$	
		ratio		*ratio*
Phytolacca americana	tris HCl	1.0	2.35	72/60 = 1.2
	tris HCl	1.0	2.0	60/52 = 1.15
Nicotiana tabacum	tris HCl	1.0	1.3	64/52 = 1.2
aurea Su/su	tris HCl	1.1	1.2	73/65 = 1.1
	tricine NaOH	1.0	1.5	71/56 = 1.3
Nicotiana tabacum	phosphate	1.0	1.0	49/42 = 1.2
NC 95	tricine NaOH	1.0	2.6	45/42 = 1.1

FIG. 4. "Titration" with ferricyanide to determine the size of the pool of primary oxidants in chloroplasts isolated from leaves of *Phytolacca americana*. T = 20°. For experimental conditions see ref. 5. No Mg added: ——; 3.3 mM $MgCl_2$ present: ----,

structural change in the grana of the chloroplast. This conclusion is based primarily on 3 observations. First, our "salt-effect" was particularly pronounced when a zwitter-ionic buffer such as tricine was used. Second, Mg^{2+} was by far more effective than alkali ions. This is in good agreement with the finding of Izawa and Good (7) that among many different cations, Mg^{2+} induced the largest volume change in chloroplasts suspended in a "low-salt" medium. Finally, the salt effect was greatly reduced after sonic disruption of the chloroplasts (see table I).

In their studies on the fluorescence kinetics of algae, Papageorgiou and Govindjee (12) have stressed already the probable influence on the fluorescence yield of structural changes in the lamellar system which accompany electron transport and/or photophosphorylation. Our results lend some support to their hypothesis.

Acknowledgments

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